EXHIBIT C

INTELLECTUAL PROPERTY INDIA

GOVERNMENT OF INDIA
MINISTRY OF COMMERCE & INDUSTRY,
PATENT OFFICE, DELHI BRANCH,
W - 5, WEST PATEL NAGAR,
NEW DELHI - 110 008.

I, the undersigned being an officer duly authorized in accordance with the provision of the Patent Act, 1970 hereby certify that annexed hereto is the true copy of the ***Application, Complete Specification*** *and* ***Drawing Sheets*** *filed in connection with Application for Patent No.199/Del/2001 dated 27th February 2001.*

Witness my hand this 9th day of June 2004.

(S.K. PANGASA)
Assistant Controller of Patents & Designs

27 FEB 2001

FORM 1
THE PATENTS ACT 1970
(39 of 1970)
APPLICATION FOR GRANT OF PATENT
(See Section 5(2), 7, 54 and 135 and rule 33A)

Received Rs. Stamps
C.P.O.D.

1. We, COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, Rafi Marg, New Delhi 110001, India, an Indian registered body incorporated under the Registration of Societies Act (Act XXI of 1860).

2. hereby declare : -
   (a) that we are in possession of an invention titled : A process for the production of 2-methylhept-4-ylisonicotinate
   (b) that the Provisional/Complete specification relating to this invention is filed with this application;
   (c) that there is no lawful ground of objection to the grant of Patent to us :

3. further declare that the inventor(s) for the said invention are :
   GAJENDRA NATH BORDOLOI, BABITA KUMARI, MANABJYOTI BORDOLOI, TARUN CH BORA AND MONOJ K ROY OF REGIONAL RESEARCH LABORATORY, JORHAT -785006, ASSAM, INDIA, ALL ARE INDIAN CITIZENS.

4. We, claim that the priority from the application(s) filed in convention countries, particulars of which are as follows :

   NOT APPLICABLE

5. We state that the said invention is an improvement in or modification of the invention, the particulars of which are as follows and of which we are the applicant:
   (a) Patent application no. :
   (b) Patent application date :

6. We state that the application is divided out of our application, the particulars of which are given below and pray that this application deemed to have been filed on ..... under section 16 of the Act.
   (a) Patent application no. :
   (b) Date of filing provisional and / or complete specification ..... and .....

7. That we are the assignee of the true and first inventor(s). :

8. That our address for service in India as follows :

   Head
   Intellectual Property Management Division, CSIR
   INSDOC Building, 14, Satsang Vihar Marg
   New Delhi 110 067

   Phone : 696 2560, 696 8819; Fax : 696 8819

9. Following declaration was given by the inventor(s) :

I / We the true and first inventor(s) for this invention declare that the applicants herein is / are my / our assignee.

Dated this 27th day of Feb. 2001

Name (in full wih expanded initials)	Signature of the true and first inventor(s)
GAJENDRA NATH BORDOLOI :	
BABITA KUMARI :	Babita Kumari
MANABJYOTI BORDOLOI :	
TARUN CH BORA :	
MONOJ K ROY :	MRoy

10. That to the best of our knowledge, information and belief the fact and matters stated herein are correct and that there is no lawful ground of objection to the grant of patent to us on this application.

11. Followings are the attachment with the application :

(a) Provisional / Complete specification (3 copies).

(b) Drawings (3 copies).

(c) Priority document(s).

(d) Statement and Undertaking on FORM - 3.

(e) Power of authority.

(f) Fee Rs 5000/- in Cheque no. : 855595 dated 22-2-2001

on State Bank of India, New Delhi Main Branch, Parliament Street, New Delhi - 110001.

We request that a patent may be granted to us for the said invention.

Dated this 27th day of Feb. 2001

Indra Dwivedy

SCIENTIST
INTELLECTUAL PROPERTY MANAGEMENT DIVISION
Council of Scientific & Industrial Research

To
The Controller of Patents
The Patent Office
New Delhi

डा० (श्रीमती) इन्द्रा द्विवेदी
DR.(MRS.) INDRA DWIBEDY
वैज्ञानिक/SCIENTIST
भा.औ.प.सं. (सी.एस.आई.आर.) I.P.M.D (C.S.I.R)
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नई दिल्ली-110067/NEW DELHI-110067

NF-9/00

FORM 2

THE PATENTS ACT-1970 (39 OF 1970)

27 FEB 2001

01 5 3 DEL 01

A Process for the Production of 2-methylheptylisonicotinate

(COMPLETE SPECIFICATION)
(See Section 10)

ORIGINAL

COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH
RAFI MARG, NEW DELHI-110001, INDIA an Indian Registered body
Incorporated under the Registration of Societies Act (XXI of 1860)

The following specification particularly describes the nature of the inventions and manner in which it is to be performed.

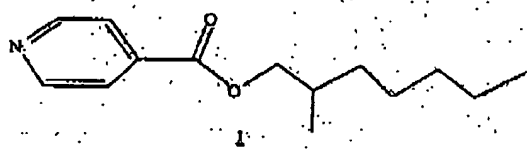
The present invention relates to a process for the production of 2-methylheptylisonicotinate. This invention particularly relates to a process for the production of an anti-fungal and antibacterial antibiotic produced by *Streptomyces* sp. 201 which exhibited marked inhibition of bacterial strains such as, *Bacillus subtilis*, *Shigella* sp., *Klebsiella* sp., *E. coli*. and *Proteus mirabilis* and antifungal activity against pathogenic test organisms, such as, *Fusarium moniliforme*, *F. semitechtum*, *F.oxysporum*, *F. solani* and *Rhizoctonia solani*.

The invention, particularly, relates to an improved process for the production of An Anti-fungal and Antibacterial Antibiotic Produced by *Streptomyces* sp. 201 of the formula 1 shown in the drawing accompanying this specification . The compound, 2-methyl heptyl isonicotinate of the formula 1 prepared by the process of the invention is found to have an yield of 2.5 mg from 500 ml of cell free culture filtrate after column chromatography and is useful as antifungal and anti-bacterial compound. The process of the present invention does not involve the use of any high boiling solvent and also does not involve the use of costly and environmentally toxic catalysts. The recovery of the solvent is also possible which makes the process simple and economic. The process of the present invention has been developed using a very non-toxic, easily isolable micro-organism identified as *Streptomyces species 201*.

The main object of the present invention, therefore, is to provide a process for the preparation of 2-methylheptylisonicotinate of the formula 1 from culture filtrate of a bacteria isolated from soil. Another object of the present invention is to provide a process for the isolation of a bacteria from soil christened as *Streptomyces species 201*, culture filtrate of which can be used to produce 2-methylheptylisonicotinate of the formula 1 and it will be commercially highly feasible and economical. Yet another object of the present invention is to provide a process for the production of 2-methylheptylisonicotinate of the formula 1 which can be used as an intermediate for the highly economical commercial production of isoniazid or isonicotinic acid hydrazide of the formula 2. Isoniazid or isonicotinic acid hydrazide of the formula 2 is an established drug for the treatment of deadly disease Tuberculosis. Still yet another object of the present invention is to provide an improved process for the production of isoniazid or isonicotinic acid 2-methylheptyl ester of the formula 1 from the culture broth of the *Streptomyces species 201* by use of a simple culture medium without using any intermediate additives. Thus the process producing this new highly active antibacterial and

antifungal compound prepared according to the process of the present invention is very simple and economical. Therefore, the simplicity of the economical and environmentally acceptable nature of the process developed by this invention makes the process commercially viable and important.

Accordingly, the present invention provides a process for production of 2-methylheptylisonicotinate of the formula 1



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which comprises: growing *Streptomyces* sp. of the kind as herein described on nutrient agar at pH 7 to 9 for a period of 6 days and subculturing in the conventional thirontons medium at a temperature in the range of 28 to 32° C for atleast 3 days, extracting culture broth with water immiscible solvent as herein described, evaporating the solvent to get crude oily substance, purifying the crude oily substance by known chromatographic methods to obtain 2-methylheptylisonicotinate.

In an embodiment of the present invention, the *Streptomyces* sp.201 may be grown on nutrient agar having following ingredients in g/L: beef extract 2.5 to 6.0, peptone 3.5 to 7.0 , potassium nitrate 0.8 to 1.4, agar 14 to 23 .

In another embodiment of the invention, the inoculation may be affected in Throntons medium having following ingredients in g/L: Dipotassium hydrogen phosphate (K_2HPO_4) 0.8 to 1.5 , potassium nitrate (KNO_3) 0.3 to 1.0, magnessium sulfate ($MgSO_4$) 0.1 to 0.4, calcium chloride ($CaCl_2.2H_2O$) 0.05 to 0.2, asparagine 0.2 to 0.7, mannitol 1.0 to 2.0, sodium chloride (NaCl) 0.05 to 0.3, ferric chloride ($FeCl_3$) 0.01 to 0.05 at pH in the range of 6.5 to 9.0.

In yet another embodiment of the invention, growing and inoculation may be affected at a temperature in the range of 28 to 32°C.

In still another embodiment of the invention, extraction of the broth may be effected by water immiscible solvents selected from a group consisting of hydrocarbons such as hexane, heptane, petroleum ether,benzene, toluene, halogenated solvents and lower acid esters such as

methyl acetate, ethyl acetate, propyl acetate. The present invention provides a process for the production of 2-methylheptylisonicotinate of the formula I and its bio-evaluation which comprises ;

a) Isolation of the Micro-organism:

A loopful of spores from slant cultures of *Steptomyces* sp.201, grown on nutrient agar was inoculated in Thronton's medium, at 30°C in a gyratory shaker, 3.3 Hz, at 200 rpm for 6 days. Both live broth and purified compound from the live broth showed very good antibacterial and antifungal activities against a number of pathogenic organisms (vide infra). The strain was collected from tea garden soils in Jorhat, Assam (26°44 N' and 94°10' E). The morphological and physiological observation of *Streptomyces* sp. 201, on nutrient agar exhibited flexible hyphae from branched aerial mycelium. Most of the mature spore chains comprised of 30 or more spores (0.7~1.0 x 1.0~1.32μm) with smooth surface and no sporongia or synnemata were observed. The vegetative mycelia were not fragmented into Bacillary and coccoid forms and the conidia were borne as sporophores indicating that the organisms belongs to the family *Streptomycetaceae* and to the genus *Streptomyces*, as evident by the production of chains of conidia in aerial hyphae.

(b) Biological activity of the live broth:

Live broth of the cultured organism *Streptomyces* sp.201 showed very good antibacterial and antifungal activities against a number of pathogenic organisms. To assess the antifungal activity of the live broth, agar cup assay method was followed. 200μl of the live broth was added into each agar cup. Zone of inhibition was found more *R. solani* (13mm), followed by *F. oxysporum* & *F. moniliformae* (12mm) *F. solani* (11 mm) and *F. semitechtum* (10 mm). The same procedure was observed more in *E. coli* (12 mm) followed by *P. mirabilis* and *Klebsiella* sp. (12 mm), *Shigella* sp. (9 mm) and *B. subtilis* (8mm). (vide table 1 below:

Table 1 : Biological activity of the live broth

Strains	Inhibition zone (mm) at 200 µg		
Antifungal activity		Antibacterial activity	
Fusarium moniliforme	12	*Bacillus subtilis*	8
F. semitechtum	10	*Shigella* sp.	9
F. oxysporum	12	*Klebsiella* sp.	11
F. solani	11	*E. coli*	12
Rhizoctonia solani	13	*Proteus mirabilis*	11

(c) Isolation of the Anti-fungal, antibacterial compound:

The bio-active compound was isolated as an oil by removing the cell mass through centrifugation followed by extraction (ethyl acetate) and purification by TLC. However, the cell mass can also be extracted with chloroform, dichloromethane, ethylene dichloride and other halogenated solvents, hexane, heptane, petroleum ether (40-60°C fraction, 60-80°C fraction or 80-100°C fraction), and other petroleum derived solvents, methyl acetate, propyl acetate, benzene, toluene or any organic solvent. The purification can be affected with column chromatography, medium pressure liquid chromatography (MPLC), high pressure liquid chromatography (HPLC) or other chromatographic methods. Purification can also be affected with fractional distillation, short path distillation etc. The compound was analyzed for $C_{14}H_{21}NO_2$ by elemental analysis and EIMS giving molecular ion at 235. The mass spectrum further gave ions at m/z 149, 126, 122, 106, 113, 99, 78 and 58. The UV spectrum of it showed λ_{max} at 220 nm. The IR spectrum of it showed absorption band at 2935, 2900, 2850, 2335, 2320, 1715, 1560 cm^{-1} indicating the presence of aromatic system with C=N- and ester group. In the ^1H NMR spectrum, two doublets of doublet with J=4 & 6 Hz, each integrating to two protons at δ 7.5 and 7.7 indicates the presence of the pyridyl system in the molecule. The multiplet signal at δ 4.22 integrating to one proton confirms the presence of the ester group. The doublet at δ 0.93 with J=7Hz and a triplet at δ 0.88 with J=7Hz, each integrating to three protons indicate the presence of two methyl groups. The DEPT experiment of the compound indicated the presence of four aromatic CH, two methyl groups, five methylenes in addition to one each of CH, quaternary aromatic carbon and carbonyl carbon. The HETCOR

NMR experiment revealed the carbon proton connectivity of the molecule. The COSY90 NMR experiment of the compound revealed the coupling between signals at δ 0.88 & 1.26, 0.93 & 1.45, 1.45 & 4.22 and 7.5 & 7.7. Based on these evidences, the structure of the compound was confirmed as 2-methylheptyl isonicotinate 1.

(C) Biological Activity:

The antibiotic activity of the compound 1 exhibited a wide range of potentialities in both pathogenic fungi and bacteria. The antibacterial activity of it was tested against dominant strains such as *Bacillus subtilis*, *E.coli*, *Shigella* sp., *Klebsiella* sp. and *Proteus mirabilis*. Maximum inhibition was recorded in *E. coli* (30 mm in diameter) and *Proteus mirabilis* (28 mm). The remaining strains of *Bacillus subtilis* and *Shigella* sp. showed an inhibition zone of 20 mm each and in *Klebsiella* sp., inhibition zone was 18 mm. The antibiotic compound exhibited promising antifungal activity when tested against dominent fungal pathogen such as *Fusarium moniliformae, F. semitechtum, F. solani, F. oxysporum and Rhizoctonia solani*. Maximum zone of inhibition was recorded in *Fusarium solani* and *F. semitechtum* (26 mm with 10 μg of the substance), and in *F. oxysporum* inhibition zone was 25 mm. Similar inhibition was also observed in *F. moniliformae* and *Rhizoctonia solani* (24 mm and 22 mm) respectively. The above antibiotic activity against dominant pathogens is the clear indication of the potentialities of the compound to find its application in both agricultural and in medical purposes.

Thus, the compound is the natural analogue of the established drug isoniazid 2 against tuberculosis (Martindale: The Extra-pharmacopeia, ed. J.E.F. Reynolds, 30 th edition, The Pharmaceutical Press, London, U.K., 1993, pp174). It may be mentioned that nearly 500,000 patients die of Tuberculosis in India every year and 5.3 million new cases of Tuberculosis are reported every year in the world (Kalia A,. 1999, A Deadly Bacilli, A report, 1999, *The Statesman*, Calcutta, 7 th April, pp7).

The details of the process disclosed in this invention have been described in the following specific examples which are provided to illustrate the invention only and therefore these should not be construed to limit the scope of the present invention.

EXAMPLE 1

a) 1. Isolation of the *Streptomyces* sp. 201:

A loopful of spores from slant cultures of *Steptomyces* sp.201, grown on nutrient agar was inoculated in Thronton's medium, at 30°C in a gyratory shaker, 3.3 Hz, at 200 rpm for 6 days. Both live broth and purified compound from the live broth showed very good antibacterial and antifungal activities against a number of pathogenic organisms such as, *Bacillus subtilis*, *Shigella* sp., *Klebsiella* sp., *E. coli*, and *Proteus mirabilis Fusarium moniliformae*, *F. semitechtum*, *F.oxysporum*, *F. solani* and *Rhizoctonia solani*. The strain was collected from tea garden soils in Jorhat, Assam (26°44' N' and 94°10' E). The morphological and physiological observation of *Streptomyces* sp. 201, on nutrient agar exhibited flexible hyphae from branched aerial mycelium. Most of the mature spore chains comprised of 30 or more spores (0.7~1.0 x 1.0~1.32µm) with smooth surface and no sporongia or synnemata were observed. The vegetative mycelia were not fragmented into Bacillary and coccoid forms and the conidia were borne as sporophores indicating that the organisms belongs to the family *Streptomycetaceae* and to the genus *Streptomyces*, as evident by the production of chains of conidia in aerial hyphae.

b) Isolation of 2-Methylheptylisoniotinate 1:

The bio-active compound was isolated as an oil by removing the cell mass through centrifugation followed by extraction with 400 ml of ethyl acetate (1:1). The solvent was removed under reduced pressure to get crude of 5 mg and was purified by silica gel G TLC (1:4 ethyl acetate hexane, ethyl acetate petroleum ether or ethyl acetate benzene). However, the Bioactive compound can also be extracted with chloroform, dichloromethane, ethylene dichloride and other halogenated solvents, hexane, heptane, petroleum ether (40-60°C fraction, 60-80°C fraction or 80-100°C fraction), and other petroleum derived solvents, methyl acetate, propyl acetate, benzene, toluene or any organic solvent. The purification can be affected with column chromatography, medium pressure liquid chromatography (MPLC), high pressure liquid chromatography (HPLC) or other chromatographic methods. Purification can also be affected with fractional distillation, short path distillation etc. The compound was analyzed by elemental analysis, EIMS, UV spectrum, IR spectrum, ^{1}H NMR spectrum, DEPT,

HETCOR and COSY90 NMR experiment to confirm the structure of the compound as 2-methylheptyl isonicotinate 1.

EXAMPLE 2:

i). Isolation of the *Streptomyces* sp. 201:

Strains were collected from tea garden soil collected within 15 KM radius of Jorhat city (26°44′ N and 94°10′ E), Assam. Screening of the strains were monitored by testing antifungal and antibacterial activity, following agar cup assay method (Gramer, 1976). Maximum activity of the bioactive molecule was observed with 6 d grown culture broth.

Melanin formation was studied in tyrosine agar medium (Waksman, 1961), containing tyrosine and yeast extract. Nitrate reduction was determined by culturing the strains in medium containing KNO_3 (0.1%,w/v), gelatin liquefaction was tested in a medium containing 12%(w/v) gelatin. Melanin formation, gelatin liquefaction and nitrate reduction was tested positive in this strain, whereas starch hydrolysis, H_2S production and citrate utilization was found negative (vide Table 2).

A loopful of spores from slant cultures of *Steptomyces* sp.201, grown on nutrient agar was inoculated into 250 ml Erlenmeyer flask containing 25 ml of Thronton's medium, consisting of (g/l): K_2HPO_4 1, KNO_3 0.5, $MgSO_4$ 0.2, $CaCl_2 2H_2O$ 0.1, asparagine 0.5, mannitol 0.1, NaCl 0.1, $FeCl_3$ 0.01, PH 7.4. The inoculated culture broth was incubated at 30°C in a gyratory shaker, 3.3 Hz, at 200 rpm (Clim-O-Shake, Adolf Kuhenen, AG), for 6 days.

(ii) Biological activity of the live broth:

Live broth of the cultured organism *Streptomyces* sp.201 showed very good antibacterial and antifungal activities against a number of pathogenic organisms. To assess the antifungal activity of the live broth, agar cup assay method was followed. 200µl of the live broth was added into each agar cup. Zone of inhibition was found more *R. solani* (13mm), followed by *F. oxysporum & F. moniliforme* (12mm) *F. solani* (11 mm) and *F. semitechtum* (10 mm). The same procedure was followed for antibacterial assay and found more in *E. coli* (12 mm) followed by *P. mirabilis* and *Klebsiella* sp. (12 mm), *Shigella* sp. (9 mm) and *B. subtilis* (8mm). (vide table 1).

2-

iii) Isolation 2-methylheptyl isonicotinate 1:

The bioactive compound was isolated by removing the cell mass through centrifugation at 4000 g for 10 min. and then the filtrate (500 ml) was extracted twice with equal volume of methyl acetate (1:1). The solvent was evaporated and the extract was dried over Na_2SO_4 under vacuum to give an oil (3.8 mg). For further purification, the crude extract was subjected to separation by thin layer chromatography (Silica gel G), using benzene: ethylacetate (4:1) as running solvent. Iodine visible compound was eluted from the silica gel with ethyl acetate, which on evaporation under reduced pressure, gave an oil, 3.2 mg. UV-λ_{max} at 220 nm; IR(cm): 2935,2900, 2850, 2335, 2320, 1715, 1560, 1540, 1510, 1462, 1380, 1290, 1085, 1060. 1H NMR (assigned by COSY90): δ 0.88 (t, J=7Hz, 3H, H-7), 0.93 (d, J=7 Hz, 3H, H-8), 1.25-1.41 (6H, overlapping signals of H-4, H-5 & H-6), 1.42 (m, 2H, H-3), 1.45 (m, 1H, H-2), 4.22 (m, 1H, H-1), 7.5 (dd, J=4&6 Hz, 2H, H-3′ & 5′), 7.7 (dd, J=4&6 Hz, 2H, H-2′ & 6′). 13C NMR (assigned by HETCOR & DEPT135): δ 11.83 (q, C-7), 14.39 (q, C-8), 23.35 (t, C-6), 24.17 (t, C-5), 29.32 (t, C-4), 30.77 (t, C-3), 39.16 (d, C-2), 68.52 (t, C-1), 129.17 (d, C-3′ & 5′), 131.23 (d, C-2′ & 6′), 132.87 (s, C-1′) and 168.09 (s, COO). MS m/z at: 235 [M^+], 149, 126, 122, 106, 113, 99, 78 and 58. Analysis: $C_{14}H_{21}NO_2$ requires C: 71.46%, H:8.99% & N:5.95%; found C: 71.44%, H: 8.91% & N: 5.96%.

iv) Biological activity :

2-Methylheptylisonicotinate 1 was bioassayed following agar cup assay method (Grammer, 1976). The antibiotic activity of the compound exhibited a wide range of potentialities in both pathogenic fungi and bacteria. The antibacterial activity of the bioactive molecule was tested against dominant bacterial strains such as *Bacillus subtilis*, *E.coli*, *Shigella* sp., *Klebsiella* sp. and *Proteus mirabilis*. Maximum inhibition was recorded in *E. coli* (30 mm in diameter) and *Proteus mirabilis* (28 mm). The remaining strains of *Bacillus subtilis* and *Shigella* sp. showed an inhibition zone of 20 mm each and in *Klebsiella* sp., inhibition zone was 18 mm. The antibitic compound exhibited promising antifungal activity when tested

against dominant fungal pathogen such as *Fusarium moniliforme, F. semitechtum; F. solani, F. oxysporum and Rhizoctonia solani.* Maximum zone of inhibition was recorded in *Fusarium solani* and *F. semitechtum* (26 mm with 10 µg of the substance), and in *F. oxysporum* inhibition zone was 25 mm. Similar inhibition was also observed in *F. moniliformae* and *Rhizoctonia solani* (24 mm and 22 mm) respectively (vide Table 2,3 & 4). The above antibiotic activity against dominant pathogens is the clear indication of the potentialities of the compound to find its application in both agricultural and medical purposes.

EXAMPLE 3:

i). Isolation of the *Streptomyces* sp. 201:

Strains were collected from tea garden soil collected within 15 KM radius of Jorhat city (26°44′ N and 94°10′ E), Assam. Screening of the strains were monitored by testing antifungal and antibacterial activity, following agar cup assay method (Gramer,1976). Maximum activity of the bioactive molecule was observed with 6 d grown culture broth.

Melanin formation was studied in tyrosine agar medium (Waksman,1961), containing tyrosine and yeast extract. Nitrate reduction was determined by culturing the strains in medium containing KNO_3 (0.1%,w/v), gelatin liquefaction was tested in a medium containing 12%(w/v) gelatin. Melanin formation, gelatin liquefaction and nitrate reduction was tested positive in this strain, whereas starch hydrolysis, H_2S production and citrate utilization was found negative.

A loopful of spores from slant cultures of *Steptomyces* sp.201, grown on nutrient agar was inoculated into 250 ml Erlenmeyer flask containing 50 ml of Thronton's medium, consisting of (g/l): K_2HPO_4 1, KNO_3 0.5, $MgSO_4$ 0.2, $CaCl_2 2H_2O$ 0.1, asparagine 0.5, mannitol 0.1, NaCl 0.1, $FeCl_3$ 0.01, PH 7.4. The inoculated culture broth was incubated at 30°C in a gyratory shaker, 3.3 Hz, at 200 rpm (Clim-O-Shake, Adolf Kuhenen, AG), for 6 days.

ii) Isolation 2-methylheptyl isonicotinate 1:

The bioactive compound was isolated by removing the cell mass through centrifugation at 4000 *g* for 10 min and then the filtrate (1000 ml) was extracted twice with equal volume of chloroform (1:1). The solvent was evaporated and the extract was dried over Na_2SO_4 under vacuum to give an oil (7.6 mg). For further purification, the crude extract was subjected to separation by thin layer chromatography (Silica gel G), using benzene: ethylacetate (4:1) as running solvent. Iodine visible compound was eluted from the silica gel with ethyl acetate, which on evaporation under reduced pressure, gave an oil, 6.4 mg. The compound was analyzed by elemental analysis, EIMS, UV spectrum, IR spectrum, ^1H NMR spectrum, DEPT, HETCOR and COSY90 NMR experiment to confirmed the structure of the compound as 2-methylheptyl isonicotinate 1.

iii) Biological activity :

2-Methylheptylisonicotinate 1 was bioassayed following agar cup assay method (Grammer, 1976). The antibiotic activity of the compound exhibited a wide range of potentialities in both pathogenic fungi and bacteria. The antibacterial activity of the bioactive molecule was tested against dominant bacterial strains such as *Bacillus subtilis, E.coli, Shigella* sp., *Klebsiella* sp. and *Proteus mirabilis*. Maximum inhibition was recorded in *E. coli* (30 mm in diameter) and *Proteus mirabilis* (28 mm). The remaining strains of *Bacillus subtilis* and *Shigella* sp. showed an inhibition zone of 20 mm each and in *Klebsiella* sp., inhibition zone was 18 mm. The antibitic compound exhibited promising antifungal activity when tested against dominant fungal pathogen such as *Fusarium monliforme, F. semitechtum, F. solani, F. oxysporum and Rhizoctonia solani*. Maximum zone of inhibition was recorded in *Fusarium solani* and *F. semitechtum* (26 mm with 10 µg of the substance), and in *F. oxysporum* inhibition zone was 25 mm. Similar inhibition was also observed in *F. moniliformae* and *Rhizoctonia solani* (24 mm and 22 mm) respectively (vide Table 2,3 & 4).

The above antibiotic activity against dominant pathogens is the clear indication of the potentialities of the compound to find its application in both agricultural and medical purposes.

Table 2: Antimicrobial activity of the purified compound

Strains	Inhibition zone (mm) at 10 µg		
Antifungal activity		Antibacterial activity	
Fusarium moniliformae	24	*Bacillus subtilis*	20
F. semitechtum	26	*Shigella* sp.	20
F. oxysporum	25	*Klebsiella* sp.	18
F. solani	26	*E. coli*	30
Rhizoctonia solani	22	*Proteus mirabilis*	28

Table 3: Antifungal activity of bioactive compound 1 and isoniazid

Organisms	MIC(µg/ml)	
	Compound 1	Isoniazid
Rhizoctonia solani	20	250
F. moniliformae	45	200
F. solani	20	300
F. semitectum	20	300
F. oxysporum	43	350

Table 4: Antibacterial activity of bioactive compound 1.

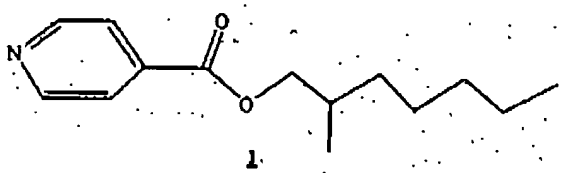
Organisms	MIC (µg/ml)	
	Compound 1	Isoniazid
Bacillus subtilis	50	150
E. coli	70	250
Arthrobacter sp.	40	150
Proteus mirabilis	40	200
Shigella sp.	60	300
Klebsiella sp.	50	250

Table 5: Antifungal and Antibacterial Activity of Bioactive Compound 1 and Isoniazid.

Fungi	MIC (µg/ml)		Bacteria	MIC (µg/ml)	
	Compound I	Isoniazid		Compound I	
R solani	20	250	*B. subtilis*	50	150
F. moniliforme	42	200	*E. coli*	70	250
F. solani	20	300	*Arthrobacter sp.*	40	150
F. semitectum	20	300	*P. mirabilis*	40	200
F. oxysporum	45	350	*Shigella sp.*	60	300
			Klebsiella sp.	50	250

We claim:

1. A process for the production of 2-methylheptylisonicotinate of the formula 1



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which comprises: growing *Streptomyces* sp. of the kind as herein described on nutrient agar at pH 7 to 9 for a period of 6 days and subculturing in the conventional throntons medium at a temperature in the range of 28 to 32^0 C for atleast 3 days, extracting culture broth with water immiscible solvent as herein described, evaporating the solvent to get crude oily substance, purifying the crude oily substance by known chromatographic methods to obtain 2-methylheptylisonicotinate.

2. A process as claimed in claim 1 wherein,, said water immiscible solvents selected from a group consisting of hydrocarbons such as hexane, heptane, petroleum ether, benzene, toluene, halogenated solvents and lower acid esters such as methyl acetate, ethyl acetate, propyl acetate.

3. A process for production of 2-methylheptylisonicotinate substantially as herein described with reference to the examples 1 to 3.

Dated this 27th day of 2001

(A VGomkale)
Scientist, IPMD
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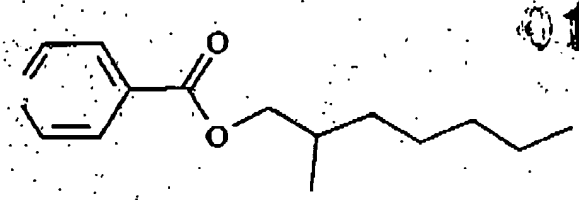

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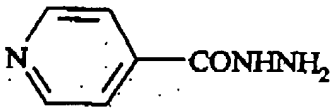

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ORIGINAL

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APPLICANTS